Claims 19-35 are pending in this application (USSN 08/312,429). The claims have been amended to more clearly recite and distinctly claim the subject matter that Applicants consider to be their invention. The claims have been amended in the interest of expediting the prosecution of this application and these amendments should in no way be construed as an acquiescence to any of the Examiner's rejections issued in U.S. Serial No. 07/837,664. Applicants reserve the right to refile claims in this or another application.

Claims 19, 21, 30, 31 and 32 have been amended to remove the word "essentially" in the phrase "essentially specific." As amended, the claims are directed to methods in which a DNA probe hybridizes "specifically" to a target chromosomally DNA. This amendment addresses and overcomes the rejection of these claims for indefiniteness under 35 U.S.C. § 112, second paragraph. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 30, 31 and 32 have been amended to indicate that the hybridization method is performed in "interphase cells, or nuclei thereof." Support for this amendment can be found throughout the specification, for example, at page 13.

Claims 29, 30 and 32 have been amended to indicate that the probes are "labeled with or labelable with" a different fluorophore or combination of fluorophores. Support for this amendment can be found throughout the specification, for example at page 15, lines 29-31, page 19, lines 2-14 and page 23, lines 13-19.

Claims 33 and 35 have been amended to correct certain informalities.

Rejection of Claims 19-25, 27-28 and 31 under 35 U.S.C. § 103

Claims 19-25, 27-28 and 31 remained rejected under U.S.C. § 103 as being unpatentable over Landegent et al. in view of Devilee et al for reasons set forth in Office Actions issued in U.S. Serial No. 07/837,664 dated March 18, 1993 and December 23, 1993.

The invention is directed to a method for detecting target chromosomal DNA *in situ* in interphase cells. The method involves combining a labeled probe DNA and competitor DNA, incubating the probe and competitor DNA with interphase cells, or nuclei thereof, under hybridization conditions and detecting the labeled probe DNA to thereby detect the target chromosomal DNA. The labeled probe DNA used in the method is characterized by containing sequences specifically hybridizable to the target chromosomal DNA and, additionally, repetitive sequences which hybridize to non-target chromosomal DNA. The competitor DNA used in the method contains the repetitive sequences which hybridize to non-target chromosomal DNA. Thus, in the claimed method, hybridization of the repetitive sequences in the DNA probe to non-target chromosomal DNA in interphase cells is suppressed by the competitor DNA.

The proper inquiry under section 103 is first whether the prior art would have suggested the claimed invention to one having ordinary skill in the art at the time the invention was made and that there was a reasonable expectation of success for the claimed invention in view of the prior art. Under section 103, "[b]oth the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure" (*Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.* 927 F.2d 1200,1207, 18 USPQ2d 1016 (Fed. Cir. 1991) quoting *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed Cir. 1988). Additionally, when a combination of references are used to establish a *prima facie* case of obviousness, it is necessary for the Examiner to present evidence that one having ordinary skill in the art would have been motivated to combine the teachings in the applied references in the proposed manner to arrive at the claimed invention. See, e.g., *Carella v. Starlight Archery*, 804 F.2d 135, 231 USPQ 644 (Fed. Cir. 1986); and *Ashland Oil, Inc. v. Delta Resins and Refractories, Inc.*, 776 F.2d 281, 227 USPQ 657 (Fed. Cir. 1985).

Landegent et al. teach *in situ* hybridization in metaphase cells using as probes one or more cosmids of the human Tg gene. Landegent et al. (at page 367, column 2, second paragraph of the Results section) describe the probes used in their method as follows:

> four different randomly chosen cosmids of the human Tg gene (of which the chromosomal localization is known, Baas et al. 1985; Landegent et al. 1985b) were used (Van Ommen et al. 1983; Baas et al. 1986)....These cosmids have not been characterized in the actual number and type of repeats present, but all gave a strong overall staining when hybridized to total human DNA under standard conditions.

Thus, the DNA probes of Landegent et al. contain unique sequences that hybridize to a target chromosomal DNA (i.e., the Tg gene) but also contain repetitive sequences that hybridize to non-target chromosomal DNA, as does the DNA probe of the method of claim 19 (and dependent claims thereof). Furthermore, in the method of Landegent et al., competitor DNA that contains the repetitive sequences is included in the hybridization procedure to inhibit hybridization of the repetitive sequences to non-target chromosomal DNA, thereby allowing for specific hybridization of the unique sequences of the DNA probe to the target chromosomal DNA. Importantly, the method of Landegent et al. differs from the claimed method in that it is performed only in *metaphase* cells not *interphase* cells, or nuclei thereof.

Accordingly, the issue with regard to the obviousness of the claimed method is whether one of ordinary skill in the art at the time the invention was made, in view of the cited references, would have been motivated to apply the *in situ* suppression hybridization method taught by Landegent et al. to interphase cells, or nuclei thereof, and whether there was a reasonable expectation of success in applying the method to interphase cells, or nuclei thereof.

When Landegent et al. itself is examined for the necessary motivation and reasonable expectation of success in applying the method described therein to interphase

cells, it is apparent that Landegent et al. teach that the method described therein is ***not*** applicable to interphase cells. In particular, Landegent et al. (at page 369, column 2, last paragraph) state:

> The method described here can extend the applicability of non-radioactive procedures since it elegantly bypasses the lower sensitivity problem, obviating subcloning of a minimal amount of unique parts required. ***When, in time***, the sensitivity has reached a level allowing the detection of small (1-2 kb) single-copy sequences on metaphase chromosomes on a routine basis, the described procedure may still retain its attractiveness for several purposes. For example: ...
> 2. Detection of chromosomal aberrations in prenatal diagnosis, for example a trisomy, in ***interphase nuclei*** in microscopic slides (Cremer et al. 1986) or through hybridization in suspension and flow cytometry. In these cases it would be favorable to use a whole panel of chromosome-specific cosmids instead of a small unique probe or a cloned alphoid sequence to ensure strong hybridization signals with great specificity, for a more reliable diagnosis [emphasis added].

The above-quoted passage contains the only reference to interphase cells, or nuclei thereof, in Landegent et al. and it is entirely future-oriented ("when, in time..."), Thus, Landegent et al. clearly teach that the sensitivity of their method is not sufficient for application to interphase cells, or nuclei thereof, at least in current form.

Accordingly, in view of the teachings of Landegent et al. alone, one of ordinary skill in the art at the time the invention was made would not have been motivated to apply the method described therein to interphase cells because Landegent et al. teach that their method, in current form, is not applicable to interphase cells. Similarly, in view of the teachings of Landegent et al. alone, one or ordinary skill in the art at the time the invention was made would not have had a reasonable expectation of success since Landegent et al. teach that the method described therein is not sensitive enough for use with interphase cells.

For the forgoing reasons, the claimed methods are nonobvious over Landegent et al. alone. We next turn to the combined teachings of Landegent et al. and Devilee et al. The Examiner rejected claims 19-25 and 27-28 under 35 U.S.C. § 103 as being unpatentable over Landegent et al. in view of Devilee et al. because

> one of ordinary skill in the art at the time the invention was made would have been motivated to use the method taught by Landegent et al. in interphase cells as taught by Devilee et al. because Devilee et al. suggest the advantages of hybridization with interphase cells and further one of ordinary skill in the art at the time the invention was made would have further had a reasonable expectation of success because Devilee et al. taught that in situ hybridization could be accomplished with interphase cells [see page 7 of the Office Action dated December 23, 1993 for USSN 07/837,6664].

As discussed previously, when an obviousness rejection is based on a combination of references, it is necessary for the Examiner to present evidence that one having ordinary skill in the art would have been motivated to combine the teachings of the applied references in the proposed manner to arrive at the claimed invention. See e.g., *Carella*, cited *supra*, *Ashland*, cited *supra*. Moreover, there must have been a reasonable expectation of success in making the claimed invention in view of the teachings of the combined references. For reasons described in detail in the following subsections, neither the requisite motivation to combine the teachings of the cited references nor the reasonable expectation of success in making the claimed invention are provided by the cited references.

A. Devilee et al. Do Not Provide the Motivation to Combine the Teachings of Landegent et al. and Devilee et al.

Devilee et al. describe an analysis of seven primary breast tumors by *in situ* hybridization to detect chromosomal abnormalities therein. The *in situ* hybridization

method taught by Devilee et al. uses interphase nucleic and chromosome specific repetitive DNA probes. More specifically, the probes of Devilee et al. are composed of "repetitive DNA sequences located predominantly in the (peri)centromeric region of one particular chromosome" (see page 5826, first sentence of Discussion). These repetitive DNA sequence probes "under proper hybridization conditions, reveal only their cognate chromosomes as distinct spots in an interphase nucleus" (see page 5826, first sentence of Discussion). Thus, the probes of Devilee et al. differ markedly from the probes of Landegent et al. More specifically, the probes of Devilee et al. contain repetitive sequences that specifically hybridize to target chromosomal DNA whereas the probes of Landegent et al. contain both unique sequences that specifically hybridize to the target chromosomal DNA of interest and repetitive sequences that hybridize to non-target chromosomal DNA.

The Examiner relies upon Devilee et al. for providing the necessary motivation to combine the teachings of Landegent et al. and Devilee et al. In particular, the Examiner points to the statement in Devilee et al. that " 'interphase cytogenetics' will prove to be a powerful tool in the study of cytogenetic heterogeneity in solid tumors" (see Devilee et al. page 5825, column 1, last two sentences of the Introduction) as providing the necessary motivation to apply the *in situ* hybridization method of Landegent et al. to interphase cells. Whatever motivation this statement may have provided to the ordinarily skilled artisan must be evaluated within the context in which the statement was made. The cited statement is set forth in the Introduction of Devilee et al. (at page 5825). The first paragraph of the Introduction describes problems in karyotyping solid tumors previously experienced in the field. Specifically, Devilee et al. state that

> [k]aryotyping procedures of solid tumors normally present difficulties such as a low mitotic index, reluctance of the cells to grow *in vitro* and poor quality metaphase figures, in which complex chromosomal rearrangements are difficult to identify (1). These drawbacks have hampered the cytogenetic characterization of extended series of solid

> tumor cases. As a consequence, it has been difficult to establish representative chromosomal aberrations or to gain insight into the existence of cytogenetic inter- or intratumor heterogeneity

Thus, in this paragraph, Devilee et al. were setting up a problem in cytogenetic characterization of solid tumors, namely that metaphase analysis was difficult to perform with solid tumors because the cells had a low mitotic index, did not grow well *in vitro* and gave poor quality metaphase figures.

In the second paragraph of the Introduction, Devilee et al. go on to describe how they have addressed this problem in tumor karyotyping. Specifically, Devilee et al. state

> It has been demonstrated (2-4) that ***ISH [in situ hybridization] of chromosome specific repetitive DNA probes***, the target sequences of which lie mainly in the (peri)centromeric region, can reveal those chromosome regions as distinct clusters of signal or spots within the interphase nucleus. Thus, ***this method***, which is fast and relatively easy, allows the counting of the number of target sites in a large number of tumor cells harvested directly from the primary cancer, without any cultivation step. We show here that the number of spots/nucleus (S/N) indicates chromosomal ploidy and/or aberrations. With regard to cellular and cytogenetic heterogeneity of solid tumors, ***the technique*** importantly allows identification of subpopulations of karyotypically aberrant cells. Analysis of seven primary breast tumors indicate that "interphase cytogenetics" will prove to be a powerful tool in the study of cytogenetic heterogeneity in solid tumors [emphasis added].

Thus, in this paragraph, Devilee et al. describe how their present study has addressed the previous problems with metaphase analysis of solid tumors. More specifically, in this paragraph, Devilee et al. describe their own successful application of ***ISH with chromosome specific repetitive DNA probes*** to tumor karyotyping in interphase nuclei. When read in context, it is apparent that the statement by Devilee et al. that " 'interphase cytogenetics' will prove to be a powerful tool in the study of cytogenetic heterogeneity in solid tumors" refers specifically to ***ISH with chromosome specific repetitive DNA probes*** as used by Devilee et al.

Devilee et al. do not teach or suggest that ISH with other types of DNA probes could be performed with interphase cells or nuclei. In particular, Devilee et al. do not teach or suggest that ISH with probes containing both unique sequences that hybridize to target chromosomal DNA and repetitive sequences that hybridize to non-target chromosomal DNA (as used by Landegent et al.) could be performed with interphase cells or nuclei. Rather, the discussion of "interphase cytogenetics" in Devilee et al. focuses solely on the use of ISH with DNA probes having repetitive sequences that hybridize to target chromosomal DNA. Thus, there is nothing in the teachings of Devilee et al. that would have directed one of ordinary skill in the art at the time the invention was made to apply the method of Landegent et al., which uses a completely different type of probe, to interphase cells or nuclei.

Recently, the Board of Patent Appeals and Interferences addressed the issue of the sufficiency of motivation required for combining the teachings of various references (*Ex parte Levengood*, 28 USPQ2d 1300 (BPAI 1993)). Specifically, the Board stated that "an Examiner cannot establish obviousness by locating references which describe various aspects of a patent applicant's invention without also providing evidence of the motivating force which would ***impel*** one skilled in the art to do what the patent applicant has done" (emphasis added). *Ex parte Levengood*, *supra*, at page 1302. Applying this standard to the quoted statement in Devilee et al., the Examiner has not met the burden of providing evidence of a motivating force sufficient to have ***impeled*** a person of ordinary skill in the art at the time the invention was made to apply the method of Landegent et al. to interphase cells, or nuclei thereof.

B. Landegent et al. Teach Away From Combining the Teachings of Landegent et al. and Devilee et al.

Not only does Devilee et al. not provide the motivation to combine the teachings therein with the teachings of Landegent et al., but additionally Landegent et al. teach away from combining the teachings of Landegent et al. and Devilee et al. In particular, with regard to application of the ISH suppression method described therein to interphase cells (once the sensitivity has been increased), Landegent et al. (at page 369, column 2, last paragraph) state that

> In these cases [i.e., detection of chromosomal aberrations in interphase nuclei], it would be favorable to use a whole panel of chromosome-specific cosmids ***instead of*** a small unique probe or ***a cloned alphoid sequence*** to ensure strong hybridization signals with great specificity, for a more reliable diagnosis [emphasis added].

Thus, Landegent et al. teach that cloned alphoid sequences are not preferred for use as probes in their method to detect chromosomal aberrations in interphase cells or nuclei.

However, ***the ISH method of Devilee et al. uses cloned alphoid sequences as DNA probes***. Devilee et al. state that their probes "belong to a class of repetitive sequences referred to as α satellite (7, 8)" (see page 5825, second column, Material and Methods section, under the heading "DNA Probes"). These α satellite sequences are also referred to in the art as alphoid sequences. For example, reference number 7, which Devilee et al. cite to describe their DNA probes, is entitled "Two subsets of human ***alphoid repetitive DNA*** show distinct preferential localization in the pericentric regions of chromosomes 13, 18 and 21" (see page 5829, second column, reference 7).

Accordingly, since Landegent et al. teach that cloned alphoid sequences are not preferred for use as DNA probes in their method to detect chromosomal aberrations in interphase cells yet Devilee et al. teach an ISH method which utilizes alphoid sequences

as DNA probes, Landegent et al. actually teach away from combining the teachings therein with the teachings of Devilee et al.

C. Landegent et al. and Devilee et al. Do Not Provide a Reasonable Expectation of Success in Making the Claimed Invention

For the reasons discussed in the previous sections, one of ordinary skill in the art at the time the invention was made would not have been motivated to combine the teachings of Landegent et al. and Devilee et al. in the proposed manner to arrive at the claimed invention. However, even if one were to assume that the requisite motivation to combine the teachings of the references were present, one of ordinary skill in the art at the time the invention was made would not have had a reasonable expectation of success in making the claimed invention in view of the teachings of the cited references for the following reasons.

At the time the invention was made, Landegent et al. taught that their ISH suppression method, while useful in metaphase cells, is not applicable to interphase cells in current form. In particular, as previously discussed, Landegent et al. taught that their method might be useful in interphase cells only "when, in time, the sensitivity has reached a level allowing the detection of small (1-2 kb) single-copy sequences on metaphase chromosomes on a routine basis" (see page 369, column 2, last paragraph). Thus, Landegent et al. taught that the sensitivity of their method would have to be improved in metaphase cells before it would be applicable to interphase cells or nuclei. However, at the time the invention was made, Devilee et al. provided no guidance to the skilled artisan as to how the sensitivity of the method of Landegent et al. could be improved so that it could be used successfully in interphase cells or nuclei. Absent such guidance, one of ordinary skill in the art would not have had a reasonable expectation of success in applying the method of Landegent et al. to interphase cells, or

nuclei thereof, since Landegent et al. taught that their method would be applicable to interphase cells or nuclei only "when, in time" the sensitivity of the method had been improved.

The Examiner argues that one of ordinary skill in the art would have had a reasonable expectation of success in applying the method of Landegent et al. to interphase cells or nuclei simply because "Devilee et al. taught that *in situ* hybridization could be accomplished with interphase cells" (see page 7 of the Office Action of December 23, 1993 in USSN 07/837,663). Applicants contend that this is too broad an interpretation of the teachings of Devilee et al. More precisely, Devilee et al. teach *in situ* hybridization with interphase cells ***only using chromosome specific repetitive DNA probes*** (i.e., alphoid sequence probes). Thus, at the time the invention was made, Devilee et al. provided no teaching or guidance to the skilled artisan as to how ISH could have been performed with interphase cells, or nuclei thereof, using other types of DNA probes. Moreover, as previously discussed, Landegent et al. specifically state that the probes taught by Devilee et al. (i.e., alphoid sequences) would not be preferred for use in their ISH method for detection of chromosomal abnormalities in interphase cells or nuclei. Accordingly, at the time the invention was made, the ordinarily skilled artisan would not have had a reasonable expectation of success in applying the ISH suppression method of Landegent et al. to interphase cells as cells taught by Devilee et al. since Landegent et al. teaches away from using the type of DNA probe used by Devilee et al. and Devilee et al. provides no guidance for using other types of DNA probes in interphase cells.

D. Conclusion

In summary, a *prima facie* case of obviousness has not been established for the claimed method in view of the combined teachings of Landegent et al. and Devilee et al. for the following reasons:

1) Devilee et al. fails to provide the necessary motivation for combining the teachings of the two references because the statement in Devilee et al. discussing the power of "interphase cytogenetics" refers only to ISH with chromosome specific repetitive DNA (i.e., alphoid sequence) probes. Devilee et al. does not teach or suggest applying ISH to interphase cells or nuclei with other types of DNA probes, in particular the probes of Landegent et al.;

2) Landegent et al. teaches away from combining the teachings of the cited references, since Landegent et al. teach that alphoid sequences are not preferred for use as probes in their ISH method in interphase cells, or nuclei thereof, yet Devilee et al. only teach ISH in interphase nuclei using alphoid sequences as probes; and

3) Even if there were a motivation to combine the teachings of Landegent et al. and Devilee et al., there would have been no reasonable expectation of success for the claimed method in view of these teachings, since Landegent et al. teach that the sensitivity of the ISH suppression technique described therein is not sufficient for application to interphase cells or nuclei but Devilee et al. provide no guidance as to how one skilled in the art could improve the sensitivity of the method of Landegent et al. so that it would be successful in interphase cells. Additionally, Landegent et al. teach that alphoid sequences are not preferred for use as DNA probes in their method yet Devilee et al. teach ISH with interphase cells or nuclei only using alphoid sequences as DNA probes and provide no guidance for use of other types of DNA probes in interphase cells or nuclei.

Rejection of Claims 26, 29-30 and 32-35 under 35 U.S.C. § 103

All other pending claims (26, 29-30 and 32-35) remained rejected under 35 U.S.C. § 103 as being unpatentable over Landegent et al. in view of Devilee et al. and other secondary references or in view of Landegent et al. and other secondary references.

Each of these claims (as originally filed or as amended herein) claim a method of *in situ* hybridization in which a labeled probe DNA containing sequences specifically hybridizable to target chromosomal DNA and, additionally, repetitive sequences which hybridize to non-target chromosomal DNA, is combined with competitor DNA that contains the repetitive sequences and used to label or detect the target chromosomal DNA in ***interphase cells, or nucleic thereof.***

The methods of claims 26, 29-30 and 32-35 are nonobvious over the teachings of Landegent et al. alone or in combination with Devilee et al. for the reasons described in detail above for rejection of claim 19-25 and 27-28 under 35 U.S.C. § 103 as being unpatentable over Landegent et al. in view of Devilee et al. The additional secondary references relied upon by the Examiner in the rejections of claim 26, 29-30 and 32-35 (i.e., Hames et al., Hopman et al. or Viegas-Pequignot et al.) do not make up for the deficiencies in Landegent et al. alone or in combination with Devilee et al. Hames et al. is relied on for teaching that short nucleic acid fragments (e.g., 50-150 nucleotides) are preferred for *in situ* hybridization (see page 8 of the Office Action dated December 23, 1993 in USSN 07/837,664). Hopman et al. is relied on for teaching labeling of two different DNA probes with distinguishable labels to allow for simultaneous detection of two different target DNA sequences (see page 7 of the Office Action dated March 18, 1993 in USSN 07/837,664). Viegas-Pequignot et al. is relied upon for teaching detection of in situ hybridization by intensified-fluorescence digital imaging microscopy (see page 10 of the Office Action dated March 18, 1993 in USSN 07/837,664).

Accordingly, none of the additional secondary references relied upon by the Examiner provide the necessary motivation to apply the method of Landegent et al. to interphase cells or to combine the teachings of Landegent et al. and Devilee et al. to arrive at the claimed invention, nor do the additional secondary references provide the necessary reasonable expectation of success for the claimed methods.

SUMMARY

In view of the above-described claim amendments and remarks, claims 19-35 are in condition for allowance. Accordingly, Applicants request reconsideration and withdrawal of the remaining rejections.

If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the examiner is urged to call the undersigned at (617) 227-7400.

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